155 Corona Avenue Pelham 65, New York

March 2, 1951

Dear Joshua,

I don't expect to do any more bacterial genetics in the immediate future, if ever, and shall be happy to see somebody going on with my experiments or anything related to them. I think Proline", B1" are not strict enough requirements (in my stocks) to be ideally suited to the study of complementary segregation, but their crossover frequencies with TL and BM are the best of several I tested. I tried this cross in at least 2 different ways and had the best results supplementing with (1) MTL and (2) PB1. You would probably want Biotin in (1) too. I got good results using either pour plates or surface inoculation, but you remember I did find a higher yield and a significant difference in composition of the MTL prototrophs when I added small am supplements of MTL. The PB, prototrophs have to be picked from pretty heavy background and carefully purified. I learned to streak out 10 strains on one plate and get good isolations. I also recommend spot-testing in rows of ten: 40 or 50 to a plate, for nutritional requirements and fermentations: for testing phage I used two rows of 10 streaks each per plate. I felt that by reducing the number of plates and doing everything in multiples of ten I could mx reduce scoring errors, a real problem when testing 200 strains for a dozen different markers. I also picked the colonies by tens, using a twelve-depression black porcelain plate, eliminating the handling of tubes altogether.

Several attempts to split up MTLPB1 in other ways were fruitless, but I didn't try it with the most prolific strains, 58-161(Proline) and W677. If I were doing it over again, I would make this cross on 4 media simultaneously: PB1, BMTL, BMB1 and PTL. Other strains either produced relatively few prototrophs or the prototrophs were aminority among mutants and background; and I'm talking about derived strains; I don't know why it should be so.

As you guessed, the cross I mentioned in my second MGB note involved BMP x TLB1. It would be reasonable to repeat the experiments with Biotin. Density of the inoculum didn't seem to have much relation to thickness of background growth, but I used surface inocula of only .02 cc of the mixture, which produced from 30 to 60 colonies per plate, the lowest number practicable for picking x 150 - 200, since many could not be lifted off satisfactorily. The frequency of complementaries from these colonies was as high as from plates with inocula 5 x as great and colony counts around 200. Some of the dilute plates had macroscopic background growth in the form of milky streaks and lines, and this is where I found the other recombinants.

I'll be on vacation in June and if we are not in New England I may get out to Cold Spring Harbor for one or two of the meetings. But the cost of the trip in time and money and my lack of close contact with the rather complex subjects would make it hardly wise for me. I have so much work to do in my field of major interest! I'll start my internship at the U.S. Marine Hospital on Staten Island July 1 if x I get my commission in the Public Health Service. That will solve a difficult financial problem and of course thedraft, and will give me all the clinical teaching I want. Besides, I may be able to do my human genetics reasarch within the Public Health Service; I'm beginning to get some concrete and, I think, original ideas for research.

My wife had a baby girl January 17 and all is going well except our sleep!

Give my regards to Esther.

Sincerely yours,

Gordon Allen